

IN THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-24 (previously cancelled).

25. (previously added) A method of modifying a lysosomal hydrolase comprising
contacting said lysosomal hydrolases with an isolated
N-acetylglucosamine-1-phosphotransferase, which has a specific activity of at least 10^6 ✓
pmol/h/mg to produce a modified lysosomal hydrolase.

26. (previously added) The method of Claim 25, further comprising purifying said
modified lysosomal hydrolase after said contacting.

27. (previously added) The method of Claim 25, wherein said
N-acetylglucosamine-phosphotransferase catalyzes the transfer of
N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the
hydrolase.

28. (previously added) The method of Claim 25, wherein said lysosomal hydrolase is
a recombinant hydrolase.

29. (Currently Amended) The method of Claim 25, wherein said lysosomal hydrolase is
selected from the group consisting of α -glucosidase, α -iduronidase, α -galactosidase A,
arylsulfatase, ~~N-acetylgalactosamine-6-sulfatase~~ N-acetylgalactosamine-6-sulfatase,
 β -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucuronidase,
Heparan N-sulfatase, N-Acetyl- α -glucosaminidase, Acetyl CoA- α -glucosaminide N-acetyl
transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A,
Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid
 β -galactosidase G_{M1} Galglioside, Acid β -galactosidase, Hexosaminidase A, Hexosaminidase

B, α -fucosidase, α -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase β -Glucosidase.

*B2
CONT.*

30. (Currently Amended) The method of Claim 25, further comprising contacting said modified lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase α -N-Acetylglucosaminidase, which catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolases and generates a terminal mannose 6-phosphate on said hydrolase.

31. (Currently Amended) The method of Claim 25, wherein said N-acetylglucosamine-1-phosphotransferase, which has a specific activity of at least 5×10^6 pmol/h/mg.

32. (Currently Amended) The method of Claim 25, wherein said N-acetylglucosamine-1-phosphotransferase, which has a specific activity of at least 12×10^6 pmol/h/mg.

33. (Currently Amended) The method of claim 25, wherein the N-acetylglucosamine-1-phosphotransferase comprises an α subunit, a β subunit, and a γ subunit; and wherein the α and β subunits are encoded by a DNA molecule comprising nucleotides 133 to 3627 of SEQ ID NO:20; and the γ subunit is encoded by a DNA molecule comprising nucleotides 296 96 to 941 of SEQ ID NO:5.

34. (Currently Amended) The method of claim 25, wherein the N-acetylglucosamine-1-phosphotransferase comprises an α subunit, a β subunit, and a γ subunit; and wherein the α and β subunits are encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 133 to 3627 of SEQ ID NO:20; and the γ subunit is encoded by a DNA molecule which hybridizes under stringent conditions

to the complement of nucleotides 296 96 to 941 of SEQ ID NO:5; wherein the combination of the α subunit, a β subunit, and a γ subunit yields a protein with the activity to catalyze the transfer of N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the hydrolase.

B2
cont.

35. (previously added) The method of Claim 25, wherein the lysosomal hydrolase is α -glucosidase.

36. (previously added) The method of Claim 25, wherein the lysosomal hydrolase is α -iduronidase.

37. (previously added) The method of Claim 25, wherein the lysosomal hydrolase is α -galactosidase A.

38. (previously added) A modified lysosomal hydrolase produced by the method of Claim 25.

39. (Currently Amended) A method of preparing a phosphorylated lysosomal hydrolase comprising contacting said lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase α -N-Acetylglucosaminidase, which has a specific activity of at least about 472,000 units/mg and which catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolases and generates a terminal mannose 6-phosphate on said hydrolase, and wherein said lysosomal hydrolase comprises a N-acetylglucosamine phosphomannose diester.

40. (previously added) The method of Claim 39, wherein said method further comprises purifying the phosphorylated lysosomal hydrolase.

41. (previously added) The method of Claim 39, wherein said lysosomal hydrolase is selected from the group consisting of α -glucosidase, α -iduronidase, α -galactosidase A, arylsulfatase, ~~N-acetylgalactosamine-6-sulfatase~~ N-acetylgalactosamine-6-sulfatase,

β -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucuronidase, Heparan N-sulfatase, N-Acetyl- α -glucosaminidase, Acetyl CoA- α -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β -galactosidase G_{M1} Ganglioside, Acid β -galactosidase, Hexosaminidase A, Hexosaminidase B, α -fucosidase, α -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase β -Glucosidase.

42. (previously added) The method of Claim 39, wherein said

N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosamindase catalyzes the removal of N-acetylglucosamine from N-acetylglucosamine phosphomannose diester to generate a terminal mannose 6-phosphate on said lysosomal hydrolase.

Claims 43 and 44. (Cancelled)

45. (previously added) The method of Claim 39, wherein the N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosamindase is encoded by a DNA molecule comprising nucleotides 151 to 1548 of SEQ ID NO:7.

46. (previously added) The method of Claim 39, wherein the N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosamindase is encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 151 to 1548 of SEQ ID NO:7.

47 (previously added) The method of Claim 39, wherein the lysosomal hydrolase is α -glucosidase.

48. (previously added) The method of Claim 39, wherein the lysosomal hydrolase is

α -iduronidase.

49. (previously added) The method of Claim 39, wherein the lysosomal hydrolase is α -galactosidase A.

50. (Currently Amended) A method of preparing a phosphorylated lysosomal hydrolase comprising:

contacting said lysosomal hydrolase with an isolated
b2
N-acetylglucosamine-phosphotransferase, which has a specific activity of at least 10^6
pmol/h/mg to produce a modified lysosomal hydrolase; and

contacting said modified lysosomal hydrolase with an isolated
N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase
 α -N-Acetylglucosaminidase, which catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolases and generates a terminal mannose 6-phosphate on said hydrolase.

51. (Currently Amended) The method of Claim 50, further comprising purifying said phosphorylated lysosomal hydrolase after said contacting with the isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase
 α -N-Acetylglucosaminidase.

52. (Currently Amended) The method of Claim 50, further comprising purifying said modified lysosomal hydrolase prior to said contacting with the isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase
 α -N-Acetylglucosaminidase.

53. (Currently Amended) The method of Claim 50, wherein said lysosomal hydrolase is selected from the group consisting of α -glucosidase, α -iduronidase, α -galactosidase A, arylsulfatase, ~~N-acetylgalactosamine-6-sulfatase~~ N-acetylgalactosamine-6-sulfatase,

β2
CONT.

β-galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucuronidase, Heparan N-sulfatase, N-Acetyl-α-glucosaminidase, Acetyl CoA-α-glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β-galactosidase G_{M1} Ganglioside, Acid β-galactosidase, Hexosaminidase A, Hexosaminidase B, α-fucosidase, α-N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase β-Glucosidase.

54. (Currently Amended) The method of Claim 50, wherein said N-acetylglucosamine-1-phosphotransferase, ~~which~~ has a specific activity of at least 5×10^6 pmol/h/mg.

55. (Currently Amended) The method of Claim 50, wherein said N-acetylglucosamine-1-phosphotransferase, ~~which~~ has a specific activity of at least 12×10^6 pmol/h/mg.

56. (previously added) The method of Claim 50, wherein the phosphorylated lysosomal hydrolase comprises at least 6% bis-phosphorylated oligosaccharides.

57. (previously added) The method of Claim 50, wherein the phosphorylated lysosomal hydrolase comprises at least 100% bis-phosphorylated oligosaccharides.

58. (previously added) The method of Claim 50, wherein the phosphorylated lysosomal hydrolase comprises at least 5 mannose 6-phosphates.

59. (Currently Amended) The method of claim 50, wherein the N-acetylglucosamine-1-phosphotransferase comprises an α subunit, a β subunit, and a γ subunit; and wherein the α and β subunits are encoded by a DNA molecule comprising

~~nucleotides 133 to 3627 of SEQ ID NO:20; and the γ subunit is encoded by a DNA molecule comprising nucleotides 296 96 to 941 of SEQ ID NO:5.~~

60. (Currently Amended) The method of Claim 50, wherein the N-acetylglucosamine-1-phosphodiester ~~α -N-Acetylglucosaminidase~~
 α -N-Acetylglucosaminidase is encoded by a DNA molecule comprising nucleotides 151 to 1548 of SEQ ID NO:7.

*B2
Cont.*

61. (Currently Amended) The method of claim 50, wherein the N-acetylglucosamine-1-phosphotransferase comprises an α subunit, a β subunit, and a γ subunit; and wherein the α and β subunits are encoded by a DNA molecule which hybridizes under stringent conditions to the complement of ~~nucleotides 133 to 3627 of SEQ ID NO:20;~~ and the γ subunit is encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 296 96 to 941 of SEQ ID NO:5; wherein the combination of the α subunit, a β subunit, and a γ subunit yields a protein with the activity to catalyze the transfer of N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the hydrolase.

62. (Currently Amended) The method of Claim 50, wherein the N-acetylglucosamine-1-phosphodiester ~~α -N-Acetylglucosaminidase~~
 α -N-Acetylglucosaminidase is encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 151 to 1548 of SEQ ID NO:7.

63. (previously added) The method of Claim 50, wherein the lysosomal hydrolase is α -glucosidase.

64. (previously added) The method of Claim 50, wherein the lysosomal hydrolase is α -iduronidase.

65. (previously added) The method of Claim 50, wherein the lysosomal hydrolase is α -galactosidase A.

66. (previously added) A phosphorylated lysosomal hydrolase produced by the method of Claim 50.

67. (New) A composition comprising the modified lysosomal hydrolase of Claim 38 and a carrier.

B2
CONT

68. (New) A composition comprising the phosphorylated lysosomal hydrolase produced by the method of Claim 50.

69. (New) The method of Claim 30, wherein said isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase has a specific activity of at least about 1000 units/mg.

70. (New) The method of Claim 30, wherein said isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase has a specific activity of at least about 472,000 units/mg.

71. (New) The method of Claim 50, wherein said isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase has a specific activity of at least about 1000 units/mg.

72. (New) The method of Claim 50, wherein said isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase has a specific activity of at least about 472,000 units/mg.
